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(54) Abstract Title
Determining allergen activity in dusts

(57) Allergen activity in dust is obtained by extracting from a dust sample at least one breakdown component of proteins or peptides; reacting the extracted at least one breakdown component with typically a colorimetric amine detection reagent such as TNBSA; and quantitatively measuring the intensity of any resulting coloration.

Allergen activity is gauged by the intensity of coloration. Further, claimed are kits for obtaining the activity. Also claimed is a method for determining the activity using a protease substrate with immobilised proteins or peptides thereon labelled with a chromogenic compound. Utility is in obtaining allergen activity of house dust mites.

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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

This print takes account of replacement documents submitted after the date of filing to enable the application to comply with the formal requirements of the Patents Rules 1995

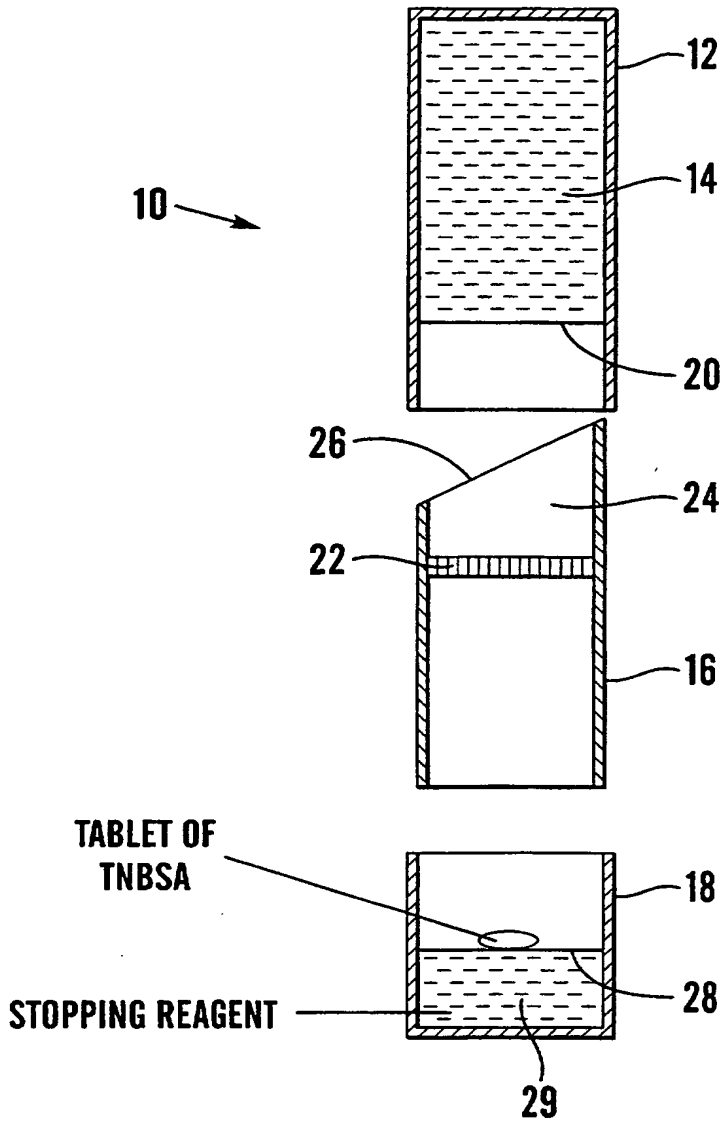
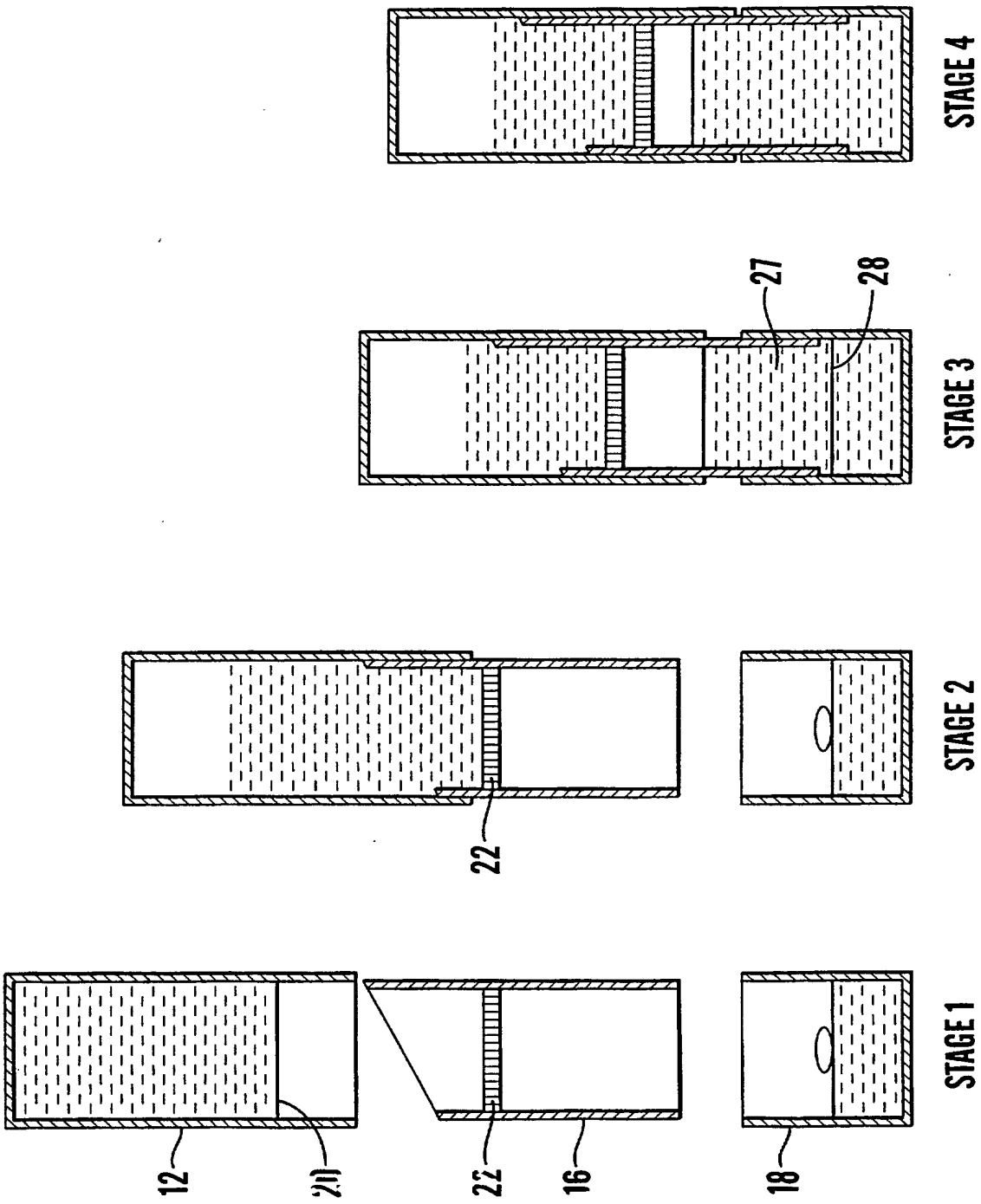


Fig. 1

Fig.2



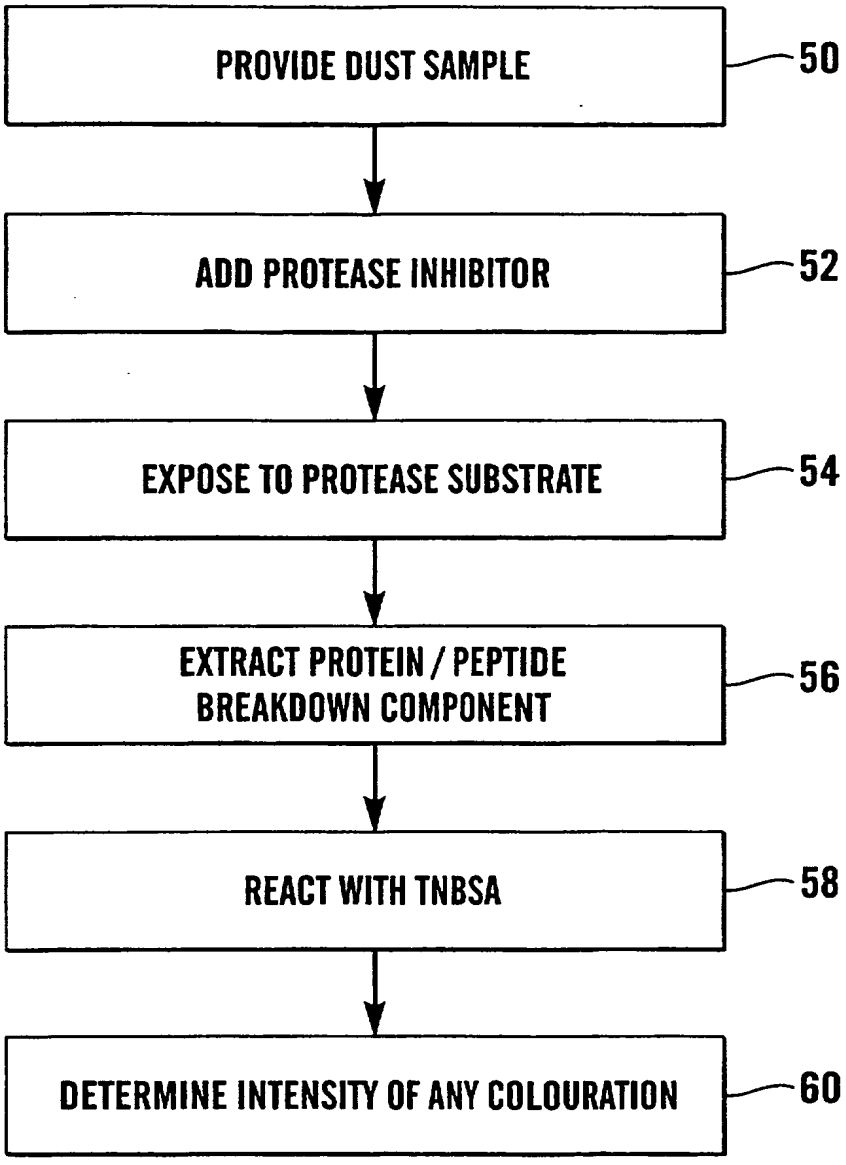


Fig.3

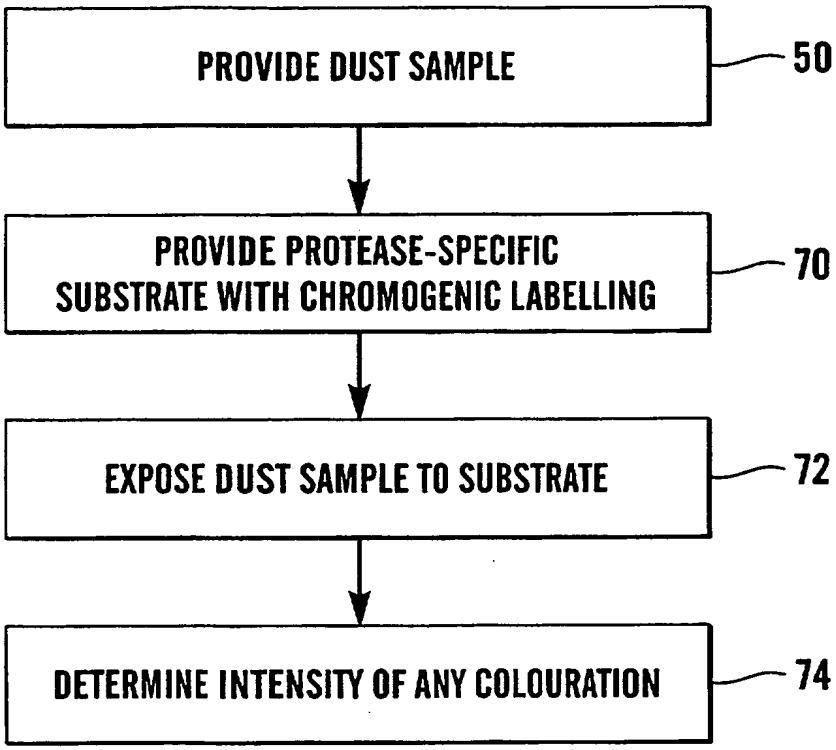


Fig.4

TITLE: ALLERGEN DETECTION

TECHNICAL FIELD

The present invention relates to allergen detection, and more particularly to a method and apparatus for indicating allergen levels in dust samples.

BACKGROUND ART

It is estimated that up to 80% of the dust particles illuminated by incident sunlight and made visible to the naked eye in a domestic environment are derived from skin. In a warm environment, dust mites feed on skin-derived dust particles, breaking it down by using proteases in their digestive system. Such proteases are found in not insignificant levels in dust mite faeces, and it is now established that it is excreted proteases which act as allergens to individuals who are liable to have an allergic response to house dust. Concentrations of excreted protease are found in relatively high levels in carpets, bedding, pillows and mattresses, all of which provide a suitable environment for dust mites to thrive.

Dust mites are not the only source of proteases

found in house dust. For example, proteases from cockroaches are also a source of allergens. Furthermore it is possible that proteases from cat saliva become airborne as the saliva dries, for example, on the cat's fur. It is likely that such proteases also act as allergens to individuals who are allergic to house dust.

It is known to test house dust in order to determine quantitatively levels of the house dust mite allergen. According to one patent, US 4,806,490, a dust sample is suspended in an aqueous-alcoholic alkali metal hydroxide solution to dissolve or leach out aromatic compounds such as guanine excreted by dust mites, and the resulting solution is mixed with an aromatic diazo compound. A reaction between the aromatic diazo compound and certain excreted aromatic compounds in the solution produces a colour change, with the intensity of the new colour being indicative of the level of excreted proteases in the house dust.

DISCLOSURE OF THE INVENTION

According to a first aspect of the present invention, there is provided a method of determining allergen activity in dust, comprising: providing a dust sample; extracting from the dust sample at least one breakdown component of proteins or peptides; reacting the extracted at least one breakdown component with a colorimetric amine detection reagent; and determining or quantitatively measuring the intensity of any resulting coloration, the allergen activity being proportioned to

the intensity of coloration.

The present applicant has appreciated that in addition to proteases, dust mites excrete the by-products of skin breakdown, including amine compounds, amino acids and relatively small chain peptides, e.g., glycylglycine. In part, the present invention is directed to detecting some of the more abundant, and in some cases chemically less complex, by-products to give an indication of the allergen concentration, rather than targeting one specific compound (e.g., guanine) or type of compounds (e.g., aromatic compounds). This will enable individuals to test particular environments, e.g., individual rooms in a domestic situation to establish that environment's propensity for inducing an allergic response.

The method may further comprise exposing the dust sample to a protease substrate, the protease substrate having immobilised thereon proteins or peptides on which protease in the dust sample may act. The protease substrate may comprise a physical support, such as a matrix or membrane. Thus, in this way, the breakdown components of proteins or peptides will at least in part be generated *in situ*. This may be useful for increasing the concentration of such components, and hence improving subsequent quantitative coloration intensity measurements. If this technique is employed, the exposure time of the dust sample to the protease substrate may need to be controlled (e.g. set at 15 minutes). It is to be noted that in such a process the allergen is

effectively being measured directly.

The method may further comprise adding a protease inhibitor to the dust sample to suppress activity of a specific protease prior to exposure to the protease substrate. In certain circumstances, it may be necessary to distinguish between dust mite protease and another protease (e.g. from cockroaches), since an individual may be more allergic to one than the other. Differentiation between the types of proteases present in the dust sample can be achieved by differential inhibition of certain specific proteases which may be present. For example, serine protease inhibitors may be used to inhibit specifically serine proteases. The serine protease inhibitors may be selected from the group consisting of organophosphates (e.g. diisopropylphosphofluoridate), sulphonyl fluorides (e.g. phenylmethylsulphonyl fluoride), coumarins (e.g. 3,4-dichloroisocoumarin) and peptide/protein inhibitors (e.g. peptide boronic acids and aprotinin, respectively). The use of serine protease inhibitors would allow dust mite allergens (e.g. cysteine proteases) to be detected more readily. On the other hand cysteine protease inhibitors may be used if dust mite allergens were to be excluded from the test. The cysteine protease inhibitors may be selected from the group consisting of peptide diazomethanes (e.g. z-Phe-Ala-CHN2), and peptide epoxides (e.g. E-64 and its derivatives), cystatins.

In one embodiment of the method, the protease

substrate is protease specific, with only a specific protease being able to act on the protein or peptide immobilised on the substrate. In this way, the protease substrate may be chosen to target a specific protease which may be present in the dust sample. If the protease is present in the sample, the specific proteins or peptides immobilised on the substrate will be broken down for subsequent detection. On the other hand, if the specific protease is absent, the proteins or peptides will remain intact and immobilised on the substrate.

The protease substrate may comprise a filter to facilitate extraction of mobile breakdown components of the proteins or peptides immobilised on the protease substrate. The filter may even act as a barrier to the passage of proteases therethrough. The breakdown components extracted from the dust sample may include amines, amino acids or peptides either from the dust sample or from the protease substrate.

The colorimetric amine detection reagent may be 2,4,6-trinitrobenzene sulphonic acid (hereinafter TNBSA).

The at least one breakdown component may be extracted by bringing the dust sample into contact with a surface active agent (surfactant). Any dust sample solid residues may be separated from the surfactant prior to reacting with the colorimetric amine detection reagent. The surfactant may be an aqueous solution comprising sodium dodecyl sulphate, possibly present in an amount of about 5 wt%. The aqueous solution may be alkaline and

may also comprise sodium hydrogen carbonate. The dust sample solid residues may be separated by filtration. Removing the solid residues facilitates accurate determination of the intensity of any coloration by reducing the amount of opaque material in the solution.

The intensity of any resulting coloration may be quantitatively determined by comparison with at least one reference colour. The comparison may be with a plurality of different colour references, each selected from the spectrum of colours or range of colour hues attainable. The different colour references may be selected to indicate at least three different kinds of allergen activity, perhaps corresponding to a macroscopic gradation such as low, medium and high activity.

The reaction mixture may be preserved by using a stopping agent, e.g., hydrochloric acid, after a pre-selected incubation or dwell time, e.g., about 2 minutes.

In order to give reproducible results, the dust sample may be of a predetermined size, e.g., by weight or by volume. The dust sample may be collected by a suction device, perhaps over a predetermined area or time. Variations in the dust sample size may be tolerated since the method represents a gross contamination test, so exact measurements of the dust samples are not necessarily essential.

In accordance with a second aspect of the invention, there is provided a method of determining allergen activity in dust, comprising: providing a dust sample;

providing a protease substrate, the protease substrate having immobilised thereon proteins or peptides labelled with a chromogenic substance; exposing the protease substrate to the dust sample under conditions whereby any protease in the dust sample may act on the immobilised protein or peptide to produce mobile breakdown components labelled with the chromogenic substance; and quantitatively measuring the intensity of any resulting coloration, the allergen activity being proportional to the intensity of the coloration.

The method may further comprise adding a protease inhibitor to the dust sample to suppress activity of a specific protease prior to exposure to the protease substrate. As before, this will enable the specific protease to be excluded from becoming actively involved in the test, allowing other protease - perhaps present in lower concentrations - to be evaluated. For example, the inhibitor may be a cysteine protease inhibitor if protease allergens other than those from dust mites are to be evaluated.

In another embodiment of the invention, the protease substrate may be protease specific, with only a specific protease being able to act on the proteins or peptides immobilised on the substrate. In this way, the test may be tailored to evaluate a specific protease, regardless of whether different kinds of protease are present in the dust sample. For example, synthetic substrates with 4-nitroaniline and 2-naphthylamine (chromophores) can be

used to distinguish between metaloproteases and aspartic proteases on the one hand (e.g. from cockroaches) and serine and cysteine proteases on the other hand (e.g. from dust mites).

The protease substrate may comprise a filter to facilitate extraction of mobile breakdown components labelled with the chromogenic substance. The filter may act as a barrier to all molecules which are larger than mobile breakdown components labelled with the chromogenic substance.

An example of a protein labelled with a chromogenic substance is azo-albumin. When reacted with a suitable protease, an azo-dye is released.

In accordance with a third aspect of the present invention, there is provided a method of determining allergen activity in dust, comprising: providing a dust sample; extracting from the dust sample at least one component selected from the group consisting of aliphatic amines and aliphatic amino acids; determining the relative concentration of the extracted at least one component; and providing an indication of allergen activity in dependence upon the relative concentration determined.

The relative concentration may be determined by employing a colour indicator sensitive to aliphatic amines and amino acids. The colour indicator may comprise TNBSA.

Any by-products of skin breakdown, particularly

aliphatic amines and aliphatic amino acids, present in the dust sample may be linked to dust mite activity. The higher the levels of the by-products in the dust sample, the higher the dust mite activity may be assumed to be. High levels of dust mite activity will produce a correspondingly high amount of protease - the allergens which are largely responsible for providing the allergic reaction to house dust in certain individuals.

In accordance with a fourth aspect of the invention, there is provided a method of determining allergen-generation propensity in dust, comprising: providing a dust sample; exposing the dust sample to a protease able to break down proteins or peptides in human skin cells; reacting the exposed dust sample with a colorimetric amine detection reagent; and quantitatively measuring the intensity of any resulting coloration, the allergen-generation propensity being proportioned to the intensity of the coloration.

An individual may want to evaluate a dust sample to see whether it might support a high level of dust mite activity, even before the allergen levels have built up to significant, detectable levels. If the dust sample contains relatively high levels of human skin cells, the protease supplied will produce breakdown components which will react with the reagent and thereby be detected by colour evaluation. By containing relatively high levels of human skin cells, the dust sampled could in theory support high concentrations of dust mite. Such

information may be a useful warning to those individuals who are allergic to dust mite protease.

The colorimetric amine detection reagent may be 2,4,6-trinitrobenzene sulphonic acid. The intensity of any resulting coloration may be quantitatively measured by comparison with at least one reference colour.

In accordance with another aspect of the present invention, there is provided apparatus for use in a domestic environment for determining indicating allergen levels. The apparatus may comprise a kit comprising a first chamber comprising a surfactant for extracting from a dust sample at least one breakdown component and of proteins and peptides; a second chamber comprising a colorimetric amine detection reagent; means for quantitatively measuring the intensity of any coloration resulting from reacting the extract-containing surfactant and the colorimetric amine detection reagent; and means for indicating relative level of allergen activity in the dust sample based on the quantitative measurement.

The apparatus may further comprise a filter for filtering dust sample solid residues from the surfactant before reacting with the colorimetric amine detection reagent, which may be TNBSA. One of the two chambers may have the capacity to receive the contents of the other chamber. Preferably, the second chamber has the capacity to hold the colorimetric amine detection apparatus and the surfactant.

The quantitative measuring means may comprise at

least one colour reference, against which the colour of the solution may be compared. The indicating means may comprise a scale, e.g., low, medium and high activity, which is linked to the intensity of any coloration measured. For example, if the colour of the solution is determined by eye as being about the same as the colour reference, this could correspond to medium allergen activity. Divergence either side of the colour reference would then correspond to low or high activity as appropriate.

The apparatus may further comprise a third chamber comprising a stopping reagent to limit the reaction between the extract-containing surfactant and colorimetric amine detection reagent, e.g. TNBSA.

BRIEF DESCRIPTION OF THE DRAWINGS

An embodiment of the invention will now be described with reference to the accompanying drawings, in which:

Figure 1 shows schematically apparatus for determining dust mite activity in accordance with the present invention;

Figure 2 shows schematically the use of apparatus shown in Figure 1;

Figure 3 is a flow chart illustrating one method of determining allergen levels according to the invention; and

Figure 4 is a flow chart illustrating another method embodying the invention.

MODES OF CARRYING OUT THE INVENTION

The apparatus 10 of figure 1 comprises three parts: an upper part 12 which contains in a first chamber 14 0.10 litres of a 0.1M solution of sodium hydrogen carbonate containing 5 wt% of sodium dodecyl sulphate; a middle part 16 which is a snug but sliding fit in both the upper part 12 and the remaining part; and a lower part 18 which contains a tablet of TNBSA and a stopping reagent of 1.0M hydrochloric acid. The solution in the first chamber 14 is sealed in the upper part 12 by a frangible seal 20. The middle part 16 comprises a filter 22 above which is provided a cup 24 for receiving a dust sample. The middle part 16 has a leading profile 26 which is pointed to facilitate breaking the frangible seal 20. A second chamber 27 is formed by the middle and lower parts. The lower part 18 includes a frangible seal 28 disposed between the tablet of TNBSA and the stopping reagent which is sealed in a third chamber 29.

The use of the apparatus 10 is now described in stages with reference to figure 2:

Stage 1 A sample of dust of predetermined size is placed in cup 24.

Stage 2 The middle part 16 is inserted into the upper part 12, such that the profile 26 ruptures the seal 20.

Stage 3 The solution in the first chamber comes into contact with the dust sample. Any chemicals including amines, amino acids and peptides present in the

dust sample are extracted and pass through filter 22 and into the second chamber where they come into contact with the tablet of TNBSA.

Stage 4 After about 2 minutes, the middle part 16 is pushed far enough into the lower part 18 to rupture seal 28, enabling the stopping reagent in the third chamber 29 to prevent further reaction. The colour of the resulting solution is compared with a colour key which is calibrated to give an indication of the level (e.g., low, medium or high) of dust mite activity in the dust sample.

Example

A dust sample was collected from an old mattress (where dust mite activity may be expected to be high), and a blank sample and test samples of GlycylGlycine in varying concentrations (20-200 micro-grams) were used as controls. The dust, blank and test samples were washed with 0.1M $NaHCO_3$ 0.5M NaCl (pH 8.3)and then tested with TNBSA of various concentrations e.g. diluted to 1 part in 10, 1 part in 50 and 1 part in 100. It was found that a dilution of 1 part in 50 was the optimum dilution for sensitivity and blank colour. Using such a dilution, the experiment yielded visual results for both the dust and all test samples, but not the blank sample. The visual results could then be assessed and compared to give an indication of dust mite activity in the old mattress.

The method used in the example may be summarised and developed with reference to Figure 3. A dust sample is

provided at step 50, possibly by using a suction device to collect dust from furniture or carpets. A protease inhibitor (e.g. serine protease inhibitor) is added at step 52) to enable a particular protease (e.g. cysteine protease) to be targeted. Next, at step 54, the dust sample is exposed to a protease substrate which is exposed to a protease substrate which is susceptible to the proteases present. Protein or peptide breakdown components from the dust sample or protease substrate are then extracted at 56 and are reacted at 58 with the colorimetric amine detection reagent (TNBSA). The presence of free amino groups causes an orange-coloured product, the intensity of which is measured at 60 to give an indication of allergen levels.

Instead of using a protease inhibitor (step 52), the protease substrate may be selected to be protease specific. In other words, the protease substrate may contain proteins or peptides which require the presence of specific proteases under evaluation before yielding detectable breakdown components.

An alternative method is illustrated in Figure 4, and again starts with the provision of a dust sample (again step 50). A specific protease substrate is provided at 70; the substrate having immobilised thereon proteins or peptides which require specific proteases before yielding breakdown components. The immobilised proteins or peptides are also labelled with a chromogenic substance. At step 72, the substrate is exposed to the

dust sample. The presence of the specific proteases in the sample will break down the immobilised proteins or peptides, releasing the chromogenic substance, causing coloration of the solution. The intensity of the coloration is measured at 74 to give an indication of allergen levels.

Instead of using a protease-specific substrate (step 70), the protease substrate may be non-specific, but still labelled with the chromogenic substance. If a specific protease is still to be targeted, this may be accomplished using protease inhibitors (step 52 of Figure 3).

CLAIMS

1. A method of determining allergen activity in dust, comprising:

providing a dust sample;

extracting from the dust sample at least one breakdown component of proteins or peptides;

reacting the extracted at least one breakdown component with a colorimetric amine detection reagent; and

quantitatively measuring the intensity of any resulting coloration, the allergen activity being proportional to the intensity of coloration.

2. A method according to claim 1, further comprising exposing the dust sample to a protease substrate, the protease substrate having immobilised thereon a protein or peptide on which protease in the dust sample may act.

3. A method according to claim 2, further comprising adding a protease inhibitor to the dust sample to suppress activity of a specific protease prior to exposure to the protease substrate.

4. A method according to claim 2, in which the protease substrate is protease specific, with only a specific protease being able to act on the protein or peptide immobilised on the substrate.

5. A method according to claim 2,3 or 4, in which the protease substrate comprises a filter to facilitate extraction of mobile breakdown components of the protein or peptide immobilised on the protease substrate.

6. A method according to any one of claims 1 to 5, in

which the breakdown components extracted from the dust sample include amines, amino acids or peptides present in the dust sample.

7. A method according to any one of claims 1 to 6, in which the colorimetric amine detection reagent is 2,4,6-trinitrobenzene sulphonic acid, (hereinafter referred to as TNBSA)

8. A method according to any one of claims 1 to 7, in which the at least one breakdown component is extracted by bringing the dust sample into contact with a surface active agent (surfactant).

9. A method according to claim 8, further comprising separating any dust sample solid residues from the surfactant prior to reacting with the colorimetric detection reagent.

10. A method according to claim 8 or 9, in which the surfactant is an aqueous solution comprising sodium dodecyl sulphate.

11. A method according to claim 10, in which the aqueous solution is alkaline.

12. A method according to claim 10 or 11, in which the aqueous solution further comprises sodium hydrogen carbonate.

13. A method according to any one of claims 1 to 12, in which the intensity of any resulting coloration is quantitatively measured by comparison with at least one reference colour.

14. A method according to claim 13, in which different

colour references are selected to indicate at least three different kinds of allergen activity.

15. A method according to any one of claims 1 to 14, further comprising preserving the reaction mixture by using a stopping agent after a pre-selected incubation period.

16. A method of determining allergen activity in dust, comprising:

providing a dust sample;

providing a protease substrate, the protease substrate having immobilised thereon proteins or peptides labelled with a chromogenic substance;

exposing the protease substrate to the dust sample under conditions whereby a protease in the dust sample may act on the immobilised protein or peptide to produce mobile breakdown components labelled with the chromogenic substance;

and quantitatively measuring the intensity of any resulting coloration, the allergen activity being proportional to the intensity of the coloration.

17. A method according to claim 16, further comprising adding a protease inhibitor to the dust sample to suppress activity of a specific protease prior to exposure to the protease substrate.

18. A method according to claim 16, in which the protease substrate is protease specific, with only a specific protease being able to act on the proteins or peptides immobilised on the substrate.

19. A method according to claim 16,17 or 18, in which the protease substrate comprises a filter to facilitate extraction of mobile breakdown components labelled with the chromogenic substance.

20. A method according to any one of claims 16 to 19, in which the intensity of any resulting coloration is quantitatively determined by comparison with at least one reference colour.

21. A method of determining allergen activity in dust, comprising: providing a dust sample; extracting from the dust sample at least one component selected from the group consisting of aliphatic amines and aliphatic amino acids; determining the relative concentration of the extracted at least one component; and providing an indication of allergen activity in dependence upon the relative concentration determined.

22. A method according to claim 21, in which the relative concentration is determined by employing a colour indicator sensitive to aliphatic amines and amino acids.

23. A method according to claim 22, in which the colour indicator comprises TNBSA.

24. A method of determining allergen-generation propensity in dust, comprising:

providing a dust sample;

exposing the dust sample to a protease able to break down proteins or peptides in human skin cells;

reacting the exposed dust sample with a colorimetric amine detection reagent; and

quantitatively measuring the intensity of any resulting coloration, the allergen-generation propensity being proportioned to the intensity of the coloration.

25. A method according to claim 24, in which the colorimetric amine detection reagent is 2,4,6-trinitrobenzene sulphonic acid.

26. A method according to claim 24 or 25, in which the intensity of any resulting coloration is quantitatively measured by comparison with at least one reference colour.

27. Kit apparatus for use in a domestic environment for indicating allergen levels, comprising a first chamber comprising a surfactant for extracting from a dust sample at least one breakdown component of proteins and peptides; a second chamber comprising a colorimetric amine detection reagent; means for quantitatively measuring the intensity of any coloration resulting from reacting the extract-containing surfactant and the colorimetric amine detection reagent; and means for indicating relative level of allergen activity in the dust sample based on the quantitative measurement.

28. Kit apparatus according to claim 27, further comprising a filter for filtering dust sample solid residues from the surfactant before reacting with the colorimetric amine detection reagent.

29. Kit apparatus according to claim 27 or 28, in which one of the two chambers has the capacity to receive the contents of the other chamber.

30. Kit apparatus according to claim 29, in which the

second chamber has the capacity to hold the colorimetric amine detection reagent and the surfactant.

31. Kit apparatus according to any one of claims 27 to 30, in which the quantitative measuring means comprises at least one colour reference, against which the intensity of any coloration may be compared.

32. Kit apparatus according to any one of claims 27 to 30, in which the indicating means comprises a scale, which is linked to the intensity of any coloration measured.

33. Kit apparatus according to any one of claims 27 to 32, further comprising a third chamber comprising a stopping reagent to limit the reaction between the extract-containing surfactant and the colorimetric amine detection reagent.

34. Kit apparatus according to any one of claims 27 to 33, in which the colorimetric amine detection reagent is 2,4,6-trinitrobenzene sulphonic acid.

35. Apparatus for use in determining allergen levels in a dust sample, comprising a protease substrate having immobilised thereon proteins or peptides labelled with a chromogenic substance, whereby any protease in the dust sample may act on the immobilised proteins or peptides to produce mobile breakdown components labelled with the chromogenic substance.

36. Apparatus according to claim 35, in which proteins labelled with chromogenic the substance comprise azo-albumin.

37. A method of determining allergen activity in dust substantially as hereinbefore described with reference to and as illustrated in the accompanying drawings.

38. Kit apparatus for determining allergen activity substantially as hereinbefore described with reference to and as illustrated in the accompanying drawings.

Application No: GB 0014054.1
Claims searched: 1-15, 21-23, 27-34
Examiner: Michael R. Wendt
Date of search: 26 October 2000

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.R): G1B (BBC, BBE)

Int Cl (Ed.7): C12Q 1/04, 1/06; G01N 31/22, 33/50, 33/52

Other: Online: EPODOC, WPI, Japio

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	EP 0174448 A2	(WERNER & M.) See Abstract.	1 & 21 at least
X	EP 0152068 A2	(WERNER & M.) See Abstract.	----"---
X	EP 0144820 A2	(WERNER & M.) See Abstract.	----"----
X	WO96/30764 A1	(VORWERK) (equivalent to US 5981287) see whole of US document	----"----
X	US 4806490	(WERNER & M.) e.g. see Claims 1 & 14. Column 5 lines 35 - 40.	----"----
X	WPI Accession No: 1992-004644 & JP 030259096 A (TORII Y.) See Abstract.		----"----

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

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